PROCESS FOR ISOLATION OF ERGOT ALKALOIDS FROM ERGOT

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FIELD OF THE INVENTION

[0001] The present invention relates to a process for the extraction and purification of ergot alkaloids and in particular to the extraction and purification of ergot peptide alkaloids from the fungi *Claviceps purpurea.*

BACKGROUND OF THE INVENTION

[0002] Ergot peptide alkaloids, also called ergopeptines, are natural products used for the manufacture of drugs. They are known to have therapeutic value themselves (e.g., ergotamine) or in their hydrogenated form such as their dihydroderivatives, e.g., dihydroergotamine, dihydroergocristine, etc. Additionally, they are known starting compounds for the partial synthesis of some semisynthetic drugs, such as nicergoline, pergolide, etc.

[0003] Ergot alkaloids of the peptide type are produced by the fungi *Claviceps purpurea*, which can be cultivated under parasitic conditions (growing on fields using rye as the host plant) or saprophytic conditions (i.e., fermentation). Although the processes used for isolating ergopeptines from field ergot and from fermentation broth share common features (i.e., similar solvents and purification techniques), they differ substantially in the nature of the starting material.

[0004] Several processes for extraction of ergot have previously been described. Individual processes, mainly that used for large-scale isolation of ergot alkaloids, differ in the solvents that are used. For example, these older processes used aqueous ethanol or methanol (see DP 47 315 and DP 697 760), newer processes used chlorinated hydrocarbons (see DE 2 113 281, DE 2 637 764, and DD 10 059), diethylether (see CS 264 880 and CS 264 881), acetone (see DE 1 695 986), methyl isobutylketone (see BE 891 421) or ethylacetate (see DE 2 949 593 and EP 22 418). Some of these solvents, however, are not currently acceptable for large scale process due to safety and ecological concerns (diethylether and chlorinated hydrocarbons being examples). Some of these solvents are additionally not selective enough to produce ergot alkaloids with enough purity to be practical for manufacturing, e.g., aqueous methanol, aqueous ethanol, and acetone.

[0005] Ergot also contains up to 30% oil and other lipids. Previous processes that used solvents that extracted other components of ergot, mainly oil, and necessarily required an operation for the separation of alkaloids from the associated lipids. However, not all the known solvents are suitable for direct elimination of lipids by liquid-liquid extraction, and therefore the isolation procedures, including concentration of the primary extracts by evaporation and dissolving the residue in another solvent, added to the complexity of the process. Additionally, the evaporation of the primary extract containing oil and other ballast components is harmful to the extracted alkaloids.

[0006] Natural ergopeptines are derivatives of lysergic acid and readily isomerize to derivatives of isolysergic acid, the so-called ergopeptinines. This fact usually complicates the isolation of ergopeptines because the primary extracts obtained from ergot always contain mixtures of ergopeptines and ergopeptinines, which makes it difficult to obtain crystalline product, thus precluding crystallization techniques, which is an otherwise efficient means of purification. Therefore, processes for large-scale isolation of ergot alkaloids, using safe and environmentally friendly solvents with simple and efficient purification operations (e.g., crystallization) are still desirable.

SUMMARY OF THE DISCLOSURE

[0007] The present invention provides a novel method of extracting ergot alkaloids from ergot.

[0008] The present method also provides a novel method of purifying the ergot extract providing ergot alkaloids in high yields and quality.

[0009] These and other advantages, which will become apparent during the following detailed description, have been obtained by the inventors' discovery that ergot can be extracted in high yield with a mixture of toluene and ethanol. Additional advantages include, but are not limited to, the ability to purify the ergot extract using relatively benign solvents and that the relatively simple process of the present invention can be scaled-up to industrial quantities.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0010] The present invention is directed to the isolation of ergot alkaloids from ergot, i.e., *Claviceps purpurea*, by a simple, effective extraction process employing relatively safe and environmentally acceptable solvents. In one embodiment of the present

invention, ergot is extracted with a toluene/ethanol mixture to form a primary extract. This primary extract can be further subjected to liquid-liquid extractions to further isolate and purify the ergot alkaloids obtained in the primary extract.

[0011] It is preferable that the toluene/ethanol extraction mixture comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, to 30% ethanol (v/v). When the concentration of ethanol is below 5%, then the extraction of ergot alkaloids from ergot is not as efficient. The upper amount of ethanol used is based on the intended selectivity of the extraction. For example, the more ethanol present the more polar ballast components that are extracted, and the more difficult it is to further process the primary extract. It is more preferable for 10-20% ethanol (v/v) to be present in the extraction mixture.

[0012] The temperature of the extraction process is advantageously not limited, because the usually unwanted isomerization of ergopeptines, which can occur at high temperature, does not impede the crystallization of the desired product. Nevertheless, temperature higher than about 50°C is not as economical. In a preferred embodiment, ergot is extracted with a toluene/ethanol mixture within a temperature range of between about 20, 25, 30, 35, 40, 45, to 50°C, with ambient temperature being preferred.

[0013] In another embodiment, the primary extract is subjected to liquid–liquid extraction using an aqueous solution of an acid. This liquid-liquid extraction of the primary extract advantageously permits separation of the generally more polar and hydrophilic alkaloids from the less polar and hydrophobic oils and lipids. Thus, liquid-liquid extraction of the primary extract with an aqueous solution containing an acid results in transferring the ergot alkaloids into the aqueous solution while leaving oil in the primary extract. The now formed aqueous extract, which contains alkaloids, can then be easily separated from the primary extract and further aids in the isolation and purification of the alkaloids. Any acid can be used with the aqueous solution. Hydrochloric acid is preferable due to the high solubility of ergopeptines hydrochlorides in aqueous hydrochloric acid solutions.

[0014] In another embodiment, an alcohol can be added to the aqueous solution to prevent or reduce the formation of an emulsion therein. It was discovered that under certain conditions, the aqueous solution can form an emulsion, which reduces the ability to isolate alkaloids. Hence, an emulsion inhibiting amount of alcohol, or similarly functioning solvent, can be added to the aqueous solution to enhance results. For

example, ethanol can be added to an aqueous solution containing hydrochloric acid used for extraction of alkaloids from the primary extract. The concentration of acid in the aqueous solution is not critical, but the solution preferably contains at least one equivalent of acid to extract the alkaloids from the primary extract quantitatively. For example, the aqueous solution of acid preferably comprises from about 30% to 60% (v/v) of water, about 70 to 40% (v/v) of ethanol and about 0.05 to 1.0% (w/w) of acid. The aqueous solution of acid more preferably comprises from about 40-50% (v/v) of water, about 60-50% (v/v) of ethanol and about 0.1-0.3% (w/w) of acid The aqueous solution of acid even more preferably comprises from about 50% (v/v) of water, about 50% (v/v) of ethanol and about 0.2% (w/w) of acid or about 40% (v/v) of water, about 60% (v/v) of ethanol and about 0.2% (w/w) of acid.

[0015] In another embodiment, the obtained aqueous extract is made alkaline (e.g., pH>7.0), thereby facilitating the alkaloid's transfer into an organic solvent during another liquid-liquid extraction. This can be done with any aqueous alkaline solution as, for example, with an aqueous solution of sodium hydroxide, more preferably 5% aqueous sodium hydroxide (w/w).

[0016] In another embodiment, the aqueous extract, after its alkalinity has been raised above 7.0, is subjected liquid-liquid extraction with toluene. The toluene extract resulting from this liquid-liquid extraction step contains practically only ergot alkaloids, and therefore it is denominated as the purified toluene extract.

[0017] In another embodiment, the purified toluene extract is partially evaporated. This partial evaporation is intended to cause formation of a crystalline product. It was found that some alkaloids, namely ergotamine and ergocristine, can be obtained as crystalline products by merely evaporating the toluene extract. The fact that the organic solvent may contain a mixture of ergopeptine and ergopeptinine does not adversely influenced the crystallization of these products, because a crystalline mixture of corresponding ergopeptine and ergopeptinine, *e.g.*, the mixture of ergotamine and ergotaminine or the mixture of ergocristine and ergocristinine, is obtained. Without being bound to any theory, it appears that the extraction and purification techniques of the present invention result in the isolation of ergot alkaloids in substantially pure form and without crystallization inhibiting impurities. Thus, even when there is a mixture of alkaloids extracted, a crystalline product can be obtained after partial evaporation of the solvent. Moreover, potential transformations of the ergot alkaloids such as isomerization of ergot

alkaloids, which can occur at high temperature, does not appear to influence the isolation and crystallization of the products obtained after evaporation of the solvent. Hence, extraction at high temperatures does not adversely affect the present process. The crystallization of alkaloids from toluene has surprisingly provides high purity products as is demonstrated in Examples 1 and 2 by comparison of the alkaloid composition of the first and the second crops.

[0018] Under certain circumstances it was found that the toluene extract can contain a mixture of alkaloids *e.g.*, a mixture of ergotoxine alkaloids, which do limit their ability to crystallize. The alkaloid composition of the toluene extract corresponds to the spectrum of alkaloids produced by the used strain of ergot. When such a mixture of ergotoxine alkaloids is present in the toluene extract, little or no crystalline product can be obtained directly from toluene. In another embodiment of the present invention, this limitation is overcome by the addition of one or more aliphatic hydrocarbon, *e.g.*, a C_5-C_8 hydrocarbon such as hexane or heptane, to the solution. Hexane is a more preferred hydrocarbon. Such crystallization technique has no effect on the alkaloid composition, but it can still produce a crystalline product free of ballast components and suitable for further use as is demonstrated in Example 3.

[0019] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments that are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

Example 1

[0020] About 20,000 kg of ergot (ergocristine strain GAL 130) were extracted under counter current conditions by a mixture of toluene and ethanol 87:13 (v/v) on a continual extractor of a carrousel type. Approximately 64 m^3 of primary extract was obtained. The primary extract was then extracted on a continual extractor Westfalia by a mixture of ethanol and water 1:1 (v/v), containing 0.2% (w/w) hydrogen chloride. Approximately 28 m^3 of aqueous extract resulted. The aqueous extract was made alkaline to about pH 7.3 by a 5% (w/w) aqueous sodium hydroxide solution and extracted with toluene on a continual extractor Westfalia. About 16 m^3 of toluene extract was received. The toluene extract was evaporated to about 1000 kg and the resulting crystalline product was filtered off, washed with toluene and dried for 3 hours in a vacuum dryer at 60°C and 50 mbar,

to obtain 152 kg of a First crop of Crude ergocristine. The mother liquors were evaporated to about 400 kg and 1500 L of technical hexane was added. The precipitated crystalline product was filtered off, washed with technical hexane and dried, to obtain 89 kg of a Second crop of Crude ergocristine.

[0021] Analytical results:

	First crop	Second crop
Assay by titration	93.1%	92.6%
Ergocristine	30.9%	20.0%
Ergocristinine	59.3%	36.6%
α-ergokryptine	2.4%	10.5%
α-ergokryptinine	3.9%	17.2%
Sum of other alkaloids	2.6%	15.7%

Example 2

[0022] About 20,000 kg of ergot (ergotamine strain GAL 404) was extracted under counter current conditions with a mixture of toluene and ethanol 84:16 (v/v) on a continual extractor of a carrousel type, and 78 m^3 of primary extract was obtained. The primary extract was extracted on a continual extractor Westfalia by a mixture of ethanol and water 6:4 (v/v), containing 0.2% (w/w) hydrogen chloride and 18 m^3 of aqueous extract resulted. The aqueous extract was made alkaline by increasing its pH to 7.3 by the addition of 5% (w/w) aqueous sodium hydroxide. It was then extracted with toluene on continual extractor Westfalia. 30 m^3 of toluene extract was received. The toluene extract was evaporated to about 1000 kg and the resulting crystalline product was filtered off, washed with toluene, and dried for 3 hours in vacuum dryer at 60°C and 50 mbar, resulting in about 181 kg of a First crop of Crude ergotamine. The mother liquors were evaporated to about 100 kg and the crystalline product was filtered off, washed with toluene, and dried, thereby obtaining 19 kg of Second crop of Crude ergotamine.

[0023] Analytical results:

	First crop	Second crop
Assay by titration	95.0%	90.6%
Ergotamine	24.7%	31.5%
Ergotaminine	71.7%	51.5%
Sum of other alkaloids	3.6%	17.0%

Example 3

[0024] About 20,000 kg of ergot (ergotoxine strain GAL 310) were extracted under counter current conditions with a mixture of toluene and ethanol 87:13 (v/v) on a continual extractor of a carrousel type. Approximately 69 m^3 of primary extract was obtained. The primary extract was extracted on a continual extractor Westfalia by a mixture of ethanol and water 1:1 (v/v), containing 0.2% (w/w) hydrogen chloride resulting in 27 m^3 of aqueous extract. The aqueous extract was made alkaline by increasing the pH of the aqueous extract to about 7.3 with 5% (w/w) aqueous sodium hydroxide. It was then extracted with toluene on a continual extractor Westfalia with about 17 m^3 of toluene extract being received. The toluene extract was evaporated to about 800 kg and 1800 L of technical hexane was added. The precipitated crystalline product was filtered off, washed with technical hexane, and dried for 3 hours in vacuum dryer at 60°C and 50 mbar obtaining 151 kg of Crude ergotoxine.

[0025] Analytical results:

Assay by titration	94.4%
Ergocornine	18.1%
Ergocorninine	29.2%
α-ergokryptine	11.7%
α-ergokryptinine	18.5%
β-ergokryptine	6.3%
β-ergokryptinine	11.6%
Sum of other alkaloids	4.6%

[0026] In this disclosure there is described only the preferred embodiments of the invention and but a few examples of its versatility. It is to be understood that the invention is capable of use in various other combinations and environments and is

capable of changes or modifications within the scope of the inventive concept as expressed herein.